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"An Experimental Evaluation on Aqueous extract of dried flower bud of *Lavanga* (*Syzygium aromaticum* Linn.) with special reference to its Analgesic activity on Wistar albino rats."

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ABSTRACT

Lavanga is one of the unique Ayurvedic drug. It is one of the frequently used drug in many preparations for pain management. Pain is an unpleasant feeling often caused by noxious stimuli. NSAIDs are the drugs used to relieve the pain and continuous usage cause adverse effects such as gastritis, nausea, vomiting, renal damage etc. to combat these situations the WHO recommended to use herbal medicine and supports the research activity on herbal drug. So, this leads to select such drug which is having analgesic action and more available drug. *Lavanga* (*Syzygium aromaticum* Linn.*)* is a drug which is having *Shulahara* property along with *Deepana, Pachana, Vatanulomana* properties. *Lavanga* used as home remedy in case of toothache and headache. Hence the present study is done to evaluate analgesic effect of Aq. Extract of *Lavanga (Syzygium aromaticum* Linn.*)* on Wistar albino rats.

Experimental study was under taken with 24 albino rats of either sex in 4 groups. 6 rats in each group namely Control group, Standard group, Test group1, Test group 2. The Eddy's hot plate method was adopted to carry-out the analgesic activity.

Analgesic activity of Aq. Extract of *Lavanga* (*Syzygium aromaticum* Linn.*)* 400mg/kg evidenced by the significant increase in pain threshold at 90th minutes compared to Control and Standard group. The study showed that the Aq. Extract of *Lavanga* (*Syzygium aromaticum* Linn.*)* at dose of 400mg/kg showed significant increase in pain threshold at 90 minutes and Aq. Extract of *Lavanga* (*Syzygium aromaticum* Linn.*)* with *Madhu* showed significant effect i.e increased pain threshold at 30th minutes.

Key words: *Lavanga*, *Syzygium aromaticum*, Aqueous extract, Analgesic activity

INTRODUCTION

The traditional medicine system based on the use of herbal preparation. 40% of the world population depends directly on the plant-based medicine. India has rich medicinal plant flora of 25,000 species out of 150 species are commercially used for extracting medicines or drug formulation. 25% of plants derivative are directly or indirectly used in modern medicine. Like eugenol which is derived from Clove oil mainly used in dentistry.

Pain is an unpleasant sensation, and emotional experience associated with actual or potential tissue damage or described in terms of such damage. Pain is mainly a protective mechanism for the body. Pain is universally understood as a pointer of disease, it is a major symptom in many medical conditions and it can interfere with person's quality of life. This brings patient towards physician. Sensation of pain involves central as well as peripheral nervous system. According to estimation of the World Health Organization's Global Burden of disease study said, Yet, every fifth person is likely to be suffering from some kind of pain. More than 20% of the world's people have headaches,>13% have migraines,7% have lower back pains, almost 5% have neck pain and 3% have osteoarthritis.[1]

The pain is treated with analgesics; analgesics are those drugs or agent which prevent pain nerve ending sensitization. Continuous administration of analgesics produces adverse effects. Hence this led to search plant origin analgesics. Because over the century's plants have been used for their medicinal values. And 80% of the population in developing countries depending on traditional medicines for primary health care.[2]

Lavanga (Syzygium aromaticum Linn.) is also called as clove, Humans have been consuming clove over a long period of time. It is known for its medicinal value. According to *Bhavaprakasha Nighantu*[3] and *Kaiyadeva Nighantu*[4] it is having *Shoolahara Karma.* Deforestation made to choose abundantly available plant, *Lavanga(Syzygium aromaticum Linn.)* is such drug which is easily and abundantly available plant. *Nighantu Adarsha* mentioned that *Lavanga Taila*

used in *Sandhishula* and *Shirashula*[5]. In *Bhaishajya Ratnavali* the formulation called *Shulavajrini vati*,[6] *Lavanga* is mentioned as one among ingredient of this preparation. This could be suggesting that it act as analgesic. This study aimed to evaluate analgesic effect of *Lavanga* where pain induced by Thermal stimuli and experimental study showed that *Lavanga* Aq. Extract has significant effect.

MATERIALS AND METHODS:

Review of literature

Lavanga (*Syzygium aromaticum* Linn.) is having Katu, Tikta Rasa, Katu Vipaka, Laghu, Snigda Guna, Sheeta Veerya. The Pushpa kalika (Dried flower bud) used as Prayojya Anga. It's Taila used in many conditions like Dantavesta Shula, Garbhini Chardi, and also having Karma such as Deepana, Pachana, Mutrala, Vedanasthapana, Vranaropana, Vrushya, Vatanulomana, Hrudya. Acharya Charaka included Lavanga (*Syzygium aromaticum* Linn) in Mukhadourgandyahara[7]. Acharya Sushruta quoted "Vaatadrute Naasti Ruja"[8] means there will not be any Ruja or Shula without the involvement of Vata. As drug Lavanga (*Syzygium aromaticum* Linn.) is having Vatanulomana Karma[9]. Vaidyamanorama mentioned it as main drug in Vatarti Shula[10]. In traditional practice Lavanga(*Syzygium aromaticum* Linn.) remedy is used in Joint pain, and Mennorhagia[11].

Madhu used in Ayurveda widely and it is one among the food, having religious significance. It is a *Pushparasa* collected by the honey bees. It is being used as *Sahapana.* It has *Medohara, Sthoulyahara, Vranashodhaka* property. The properties of *Madhu* according to Ayurveda such as *Madhura Rasa, Kashaya Anurasa, Ushna Veerya, Katu Vipaka, Tridoshahara.* And has *Yogavahi Guna*[12] which enhances the action of another drug.

Pentazocine it is a narcotic drug which mainly acts on Central nervous system. It is used to treat moderate to severe pain. Also used as anesthesia for surgery. It acts on K- opioid receptors and blocking the μ opioid receptor[13].

Procurement of drug: The drug dried flower bud of *Lavanga* is procured from Market and it is authenticated by faculty of department of PG studies in Dravyaguna.

Extraction of drug: The Aqueous

extract of *Lavanga* (Syzygium aromaticum Linn.) prepared by using hot infusion method in the ratio of 450:1000 that is, drug: distilled water. The mixture was heated for 1 hour/ day for 3 days with intermittent shaking. And then extract was dried under shade.

Procurement of animals: 24 Wistar albino rats weighing 150-200gm were taken for analgesic activity carried through eddy' hot plate method at 50^0C temperature. Experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC/HSKCOP/March 2020/PG 13) held at H.S.K College of Pharmacy Bagalkot.

Location of the study- The Aq. extract of dried flower bud of *Lavanga* and animal experimental study carried out in H.S.K College of Pharmacy, Bagalkot.

Grouping- The rats were divided into 4 groups 6 in each group. Marking had been done on tail for identification

Control group	Distilled water
Standard group	Standard drug (Pentazocine)
Test group 1	*Lavanga* (*Syzygium aromaticum Linn)* Aq. Extract
Test group 2	*Lavanga* (*Syzygium aromaticum Linn)* Aq. extract with *Madhu* (honey)

Mode of administration: administration of test drug through 1ml disposable Insulin syringe fitted with infant feeding tube introduced to the esophagus to facilitate its smooth entry inside the throat.

Dosage: The moderate dose will be given that is 1/5th dose of LD_{50} (2000 mg/kg)

Test-1 group: 400mg/kg given orally

Test -2 group:400mg/kg with 1ml of *Madhu* given orally

Standard group:8mg/kg (Pentazocine) given intraperitoneal

Control group: distilled water

Experimental procedure[14]: 4 Groups of 24 Healthy Wistar Albino rats of either sex weighing 150 to 200gm. Each group with 6 animals kept in separate cage and numbered. The animals are placed on the hot

plate (maintaining 55^0c heat) and the reaction time is noted by stop watch until either jumping or paw licking occurs is recorded before and after 0, 15th, 30th, 60th, 90th, 120th minutes following administration of test sample and standard drug.

Parameters for experimental study: Paw licking and Jumping response of rat when placed on Hot plate.

RESULTS:

Experimental Study: In-vivo study of Analgesic activity

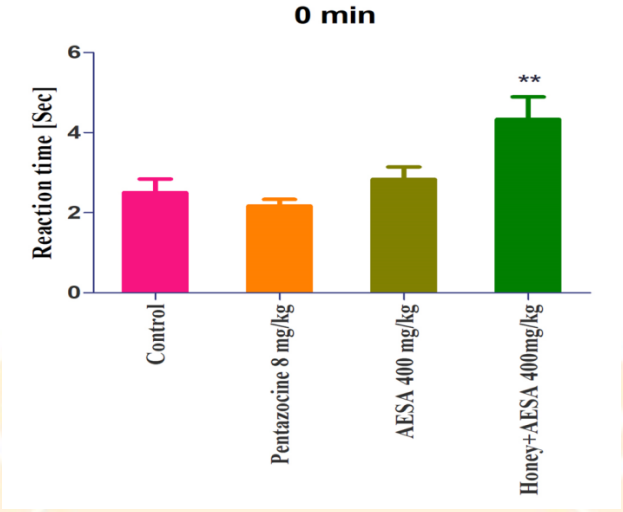
Observation of Test drug, Standard, and Control group on pain threshold on hot plate At 0 minutes the Test group-2(Aq. Extract of *Lavanga* with *Madhu*) group showed more reaction time i.e increased pain threshold compared to control, standard and Test group-1(Aq extract of dried flower bud of *Lavanga*). At 15 minutes, the more reaction time shown by Test group-2 compared to other groups. At 30 minutes, the Test group-1 showed moderate reaction time and Test group-2 and Standard group(Pentazocine) showed more reaction time compared to Control group. At 60 minutes, the Test group-1 and Standard group both showed more reaction time compared to Control group. At 90 minutes, the Test group-1showed more reaction time compared to other group. At 120 minutes, the Test group-1showed more reaction time and Test group-2 showed moderate reaction time compared to Control group. Madhu act as Yogavahi (bioenhancer) enhance the action of *Lavanga* and results in increased pain threshold time of Test group-2 compared to Test group-1.

Statistical Analysis of Analgesic activity:

Showing analgesic effect of the test drug at 0 minutes

Group	SD	SE	Mean	P value	F value
Control group	0.8367	0.3416	2.500	0.0027	6.65
Test group 1	0.7528	0.3073	2.833		
Test group 2	1.366	0.5578	4.333		
Standard group	0.4082	1.667	2.167		

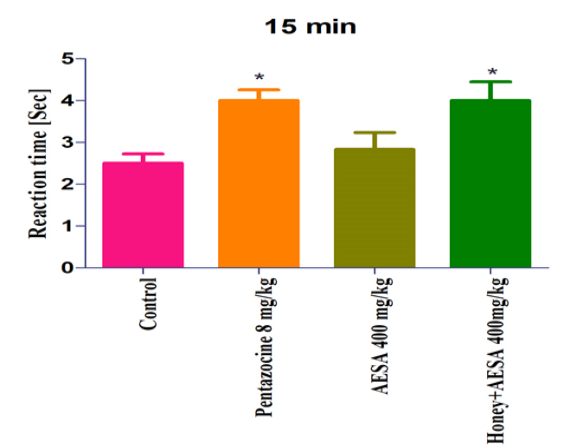
Graph. no1: Paw lick or jumping response at 0 minutes



Showing analgesic effect of the test drug at 15 minutes

Group	SD	SE	Mean	P value	F value
Control group	0.5477	0.2236	2.500	<0.0087	5.116
Test group 1	0.9832	0.4014	2.833		
Test group 2	1.095	0.4472	4.000		
Standard group	0.6325	0.2582	4.000		

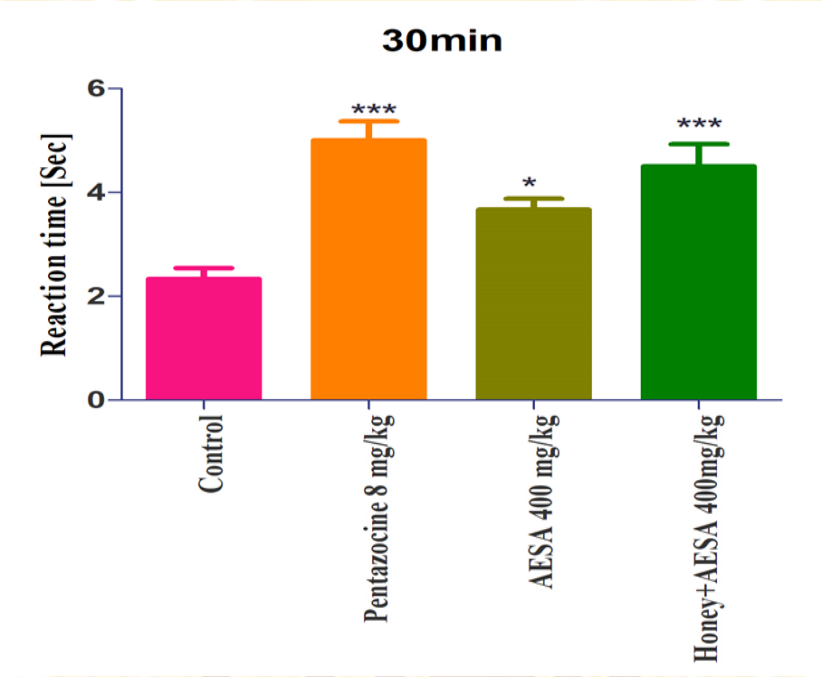
Graph. no 2: Paw licking or jumping response at 15 minutes



Showing analgesic effect of the test drug at 30 minutes.

Group	SD	SE	Mean	P value	F value
Control group	0.5164	0.2108	2.333	<0.0001	13.40
Test group 1	0.5164	0.2108	3.667		
Test group 2	1.049	0.4282	4.500		
Standard group	0.8944	0.3651	5.000		

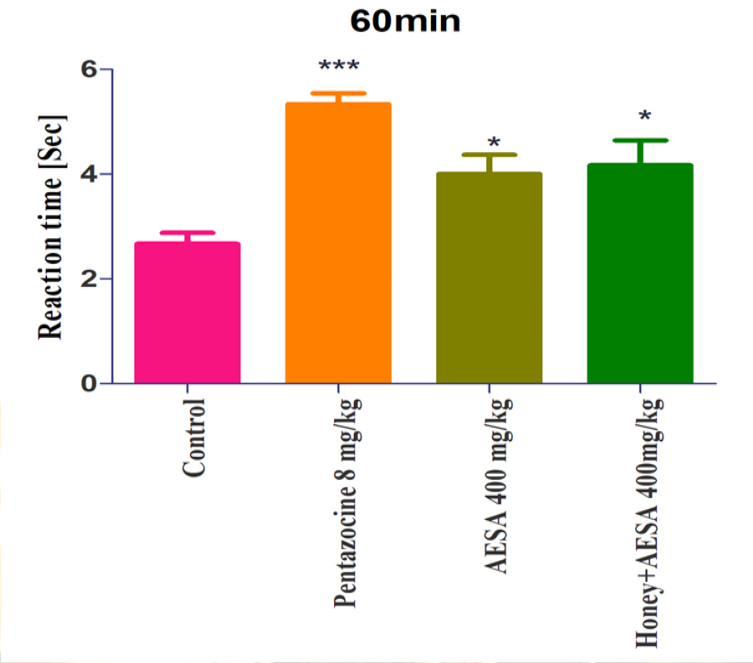
Graph no 3: Paw licking or jumping response at 30 minutes



Showing analgesic effect of the test drug at 60 minutes.

Group	SD	SE	Mean	P value	F value
Control group	0.5164	0.2108	2.667	0.0002	10.60
Test group 1	0.8944	0.3651	4.000		
Test group 2	1.169	0.4773	4.167		
Standard group	0.5164	0.2108	5.333		

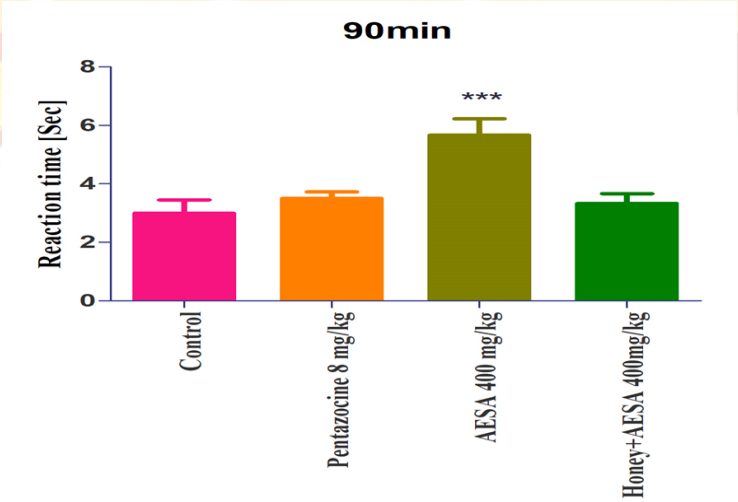
Graph. no 4: Paw licking or jumping response at 60 minutes



Showing analgesic effect of the test drug at 90 minutes.

Group	SD	SE	Mean	P value	F value
Control group	1.095	0.4472	3.000	0.0007	8.747
Test group 1	1.366	0.5578	5.667		
Test group 2	0.8165	0.3333	3.333		
Standard group	0.5477	0.2236	3.500		

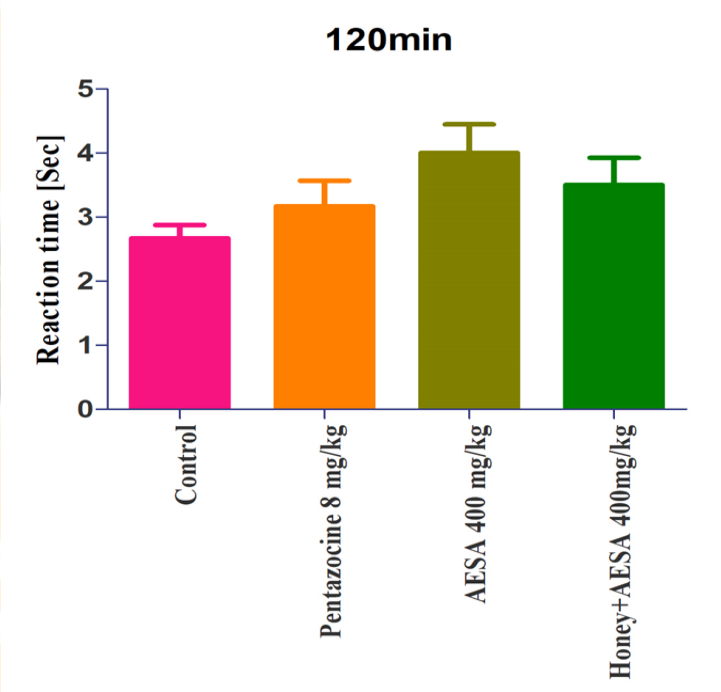
Graph. no 5: Paw licking or jumping response at 90 minutes



Showing analgesic effect of the test drug at 120 minutes.

Group	SD	SE	Mean	P value	F value
Control group	0.5164	0.2108	2.667	0.1273	2.138
Test group 1	1.095	0.4472	4.000		
Test group 2	1.049	0.4282	3.500		
Standard group	0.9832	0.4014	3.167		

Graph. no 6: Paw licking or jumping response at 120 minutes



Results of Analgesic activity:

Showing Significant activity in various time intervals.

Effect of Aq. extract of *Lavanga* (*Syzygium aromaticum* Linn) dried flower bud on pain threshold by thermal pain induced by Eddy's hot plate.

Groups	Recording of pain threshold at various time intervals after drug administration					
	0 min	15 min	30 min	60 min	90 min	120 min
Control	2.5±0.34	2.50± 0.22	2.33 ± 0.21	2.6± 0.21	3.0±0.45	2.6± 0.21

Standard Group Pentazocine 8mg/kg	2.17± 0.16	4.00± 0.25*	5.00± 0.36***	5.33± 0.21***	3.5± 0.22	3.16± 0.4
Test Group-1 AESA 400mg/kg	2.83± 0.30	2.83± 0.40	3.66± 0.21 *	4.00± 0.36*	5.66± 0.55***	4 ± 0.44
Test Group-2 AESA400mg/kg+ *Madhu*	4.33± 0.55**	4.00± 0.44*	4.5± 0.42***	4.16± 0.47*	3.3± 0.33	3.5± 0.42

All the values are expressed as Mean± SEM, n=6, One way Analysis of Variance (ANOVA) followed by multiple comparison dunnett test. *p<0.05, **p<0.01, ***p<0.001 as compared to the control group

Results:

Analgesic activity was performed by Eddy's hot plate method. In this study the standard drug pentazocine (8mg/kg) showed significant analgesic activity (p<0.05 – p<0.001) by increase in reaction time (sec) 4.00± 0.25, 5.00± 0.36 and 5.33±0.21 at 15, 30 and 60 minutes respectively as compared to control group. At the same the aqueous extract of dried flower bud of Lavanga showed significant analgesic activity (p<0.05 –p<0.001) by increase in reaction time (sec) 3.66± 0.21, 4.00± 0.36 and 5.66± 0.55 at 30, 60 and 90 minutes respectively as compared to control group. In contrast the aqueous extract of dried flower bud of Lavanga along with Madhu showed significant analgesic activity (p<0.05 –p<0.001) by increase in reaction time (sec) 4.33± 0.55, 4.00± 0.44, 4.5 ± 0.42 and 4.16 ± 0.47 at 0, 15, 30 and 60 minutes respectively as compared to control group.

DISCUSSION:

Experimental study: In-vivo study

The experimental study was carried out to evaluate the analgesic effect of drug *Lavanga* (*Syzygium aromaticum* Linn.) aqueous extract. Efficacy of test drug and test drug with *Madhu* has been compared with control group to establish effect of test drug and test drug with *Madhu.*

Several methods were used for assessing the analgesic activity. Eddy's hot plate method was selected to assess the analgesic effect on central mechanism.

In present study Eddy's hot plate method was employed and test drug shown significant analgesic action on central mechanism.

Discussion on Result:

Effect of aqueous extract of *Lavanga* (*Syzygium aromaticum* Linn) dried flower bud on pain threshold by thermal pain induced by Eddy's hot plate.

The standard group showed significant action at 15th minutes then it peaked at 30th and 60th minutes after that it showed insignificant analgesic action when compared to control group. The Test group-1 showed significant at 30th and 60th minute and It peaked at 90th minute, after that also it showed significant analgesic action with gradual decrease in reaction time. Test group-2 showed its increased reaction time at 0 minutes and reached peak at 30th minutes. *Madhu* showed early reaction time means increased pain threshold and helped in better absorption and bioavailability of drug this leads to faster drug action. This inferred that the Aq. Extract of dried flower bud of *Lavanga* act as analgesic for longer period and aq. Extract of dried flower bud of *Lavanga* with *Madhu* act as analgesic for short period.

Probable mode of action:

The dried flower bud of *Lavanga(Syzygium aromaticum* Linn*)* having *Katu, Tikta Rasa*, *Sheeta Veerya, Madhura Vipaka. Vata* is a causative factor for *Shula*. This drug has *Vatanalomana* action and *Madhura Vipaka* so helps to relieve *Vataja Shula.* The *Katu* and *Tikta Rasa* helps in *Kapha Shoshana*. And it relieves pain due to *Kapha Dosha*. The *Sheeta Veerya* helps to relieve *Pittaja Shula*. *Madhura Vipaka* helps to relieve *Vataja Shula*. According to *Madanapala Nighantu Lavanga* is having *Tridoshahara*. Hence it may also help in *Tridoshaja Shula*. As it is having *Deepana, Pachana* it helps in *Amaja Shula*. *Lavanga* has *Shulahara* action which is mentioned by *Bhavaprakasha Nighantu* and *Kaiyadeva Nighantu.* And *Lavanga* is one among the *Shulahara dravya*. It is mentioned in *Shulavajrini vati* it inferred that *Lavanga (Syzygium aromaticum* Linn*)* has *shulahara* property.

The experimental study of Aq. Extract of dried flower bud of *Lavanga (Syzygium aromaticum* Linn*)* showed significant analgesic action on Wistar albino rats.

CONCLUSION:

The Aq. Extract of *Lavanga* (Syzygium aromaticum Linn.) possesses analgesic activity. And this justifies the use of

this plant as herbal remedy against pain. Aq. Extract of dried flower bud of *Lavanga* showed significant analgesic activity in 60th and 90th minutes. And Aq. extract of *Lavanga* with *Madhu* showed significant effect in short time i.e within 15th to 60th minutes. This shows that *Madhu* helps in better absorption, enhance the bioavailability of *Lavanga* and results in faster drug action.

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